

# Discovery Research

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## *In Vitro* Biology Capabilities



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# *In Vitro* Biology



<b>Expertise</b>	A group of dedicated scientists with overall expertise in Biochemistry, Molecular Biology and Cell Biology
<b>Drug target class</b>	Encompassing drug target class: GPCRs, Nuclear Receptors, Monoamine transporters, ion channels and enzymes
<b>Proficiency</b>	Assay Development and Validation, Screening and Profiling of Compounds, and Diverse Mechanistic Studies of Lead/ Lead Like Molecules

# Protein Expression, Purification and Interaction Services



## Cloning And Expression

- Human cDNA Synthesis and Cloning
- Expression of Recombinant Proteins in *E. coli*, Baculovirus and Mammalian Cells.



## Protein Purification

- Purification and Characterization of Recombinant Proteins using affinity tag



## Protein – Protein Interactions

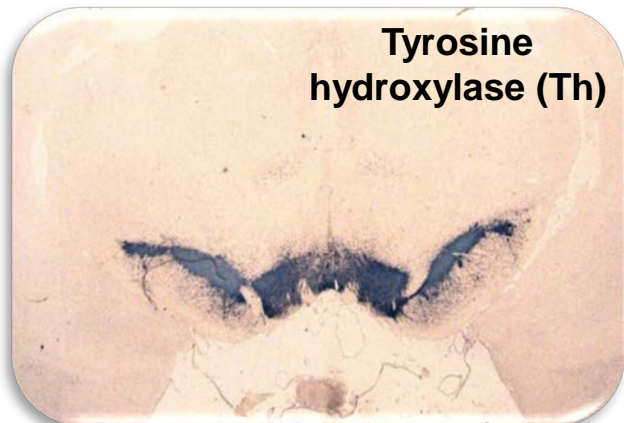
- Yeast Two-Hybrid System and other yeast based assays
- His Tag Pull Down

# Protein Localization

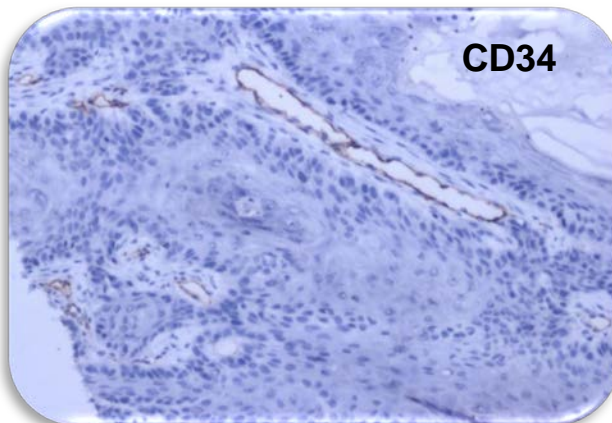


## Protein Localization - Immunohistochemistry

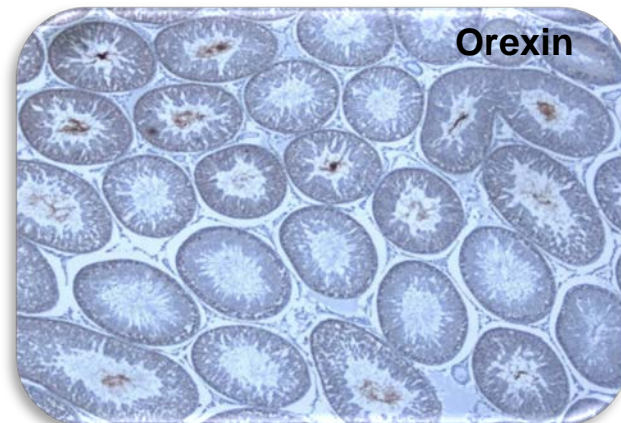
**Tyrosine hydroxylase (Th)**



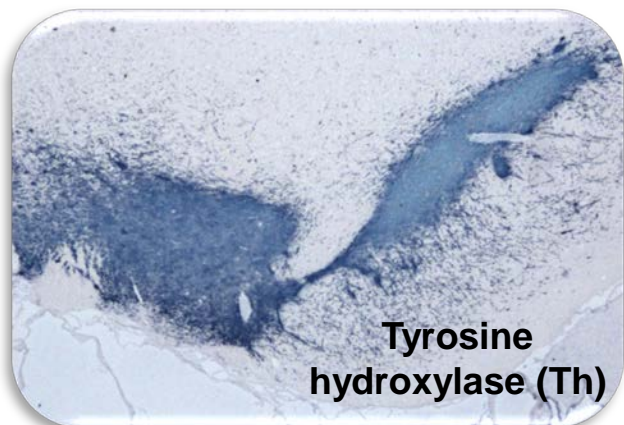
**CD34**



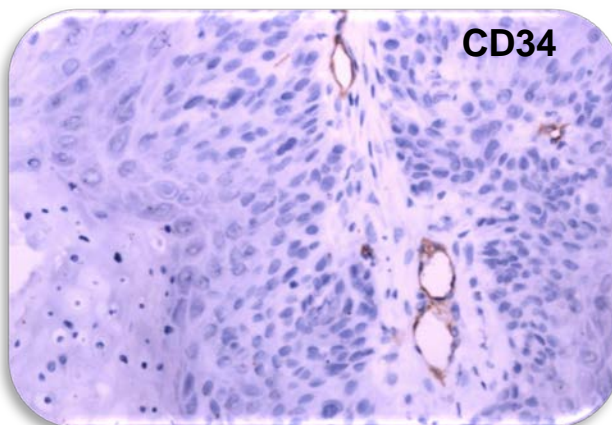
**Orexin**



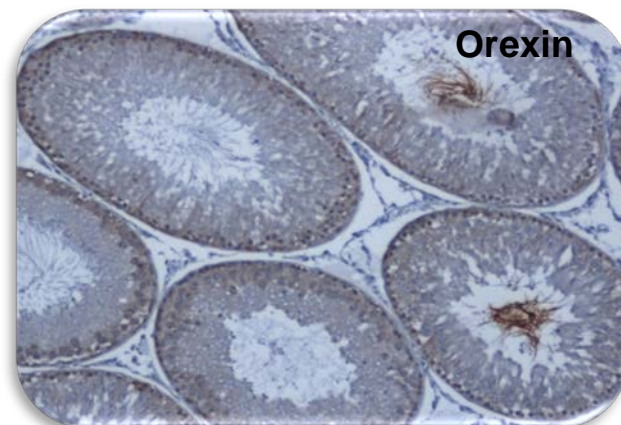
**Tyrosine hydroxylase (Th)**



**CD34**



**Orexin**



# Cell line and cell culture



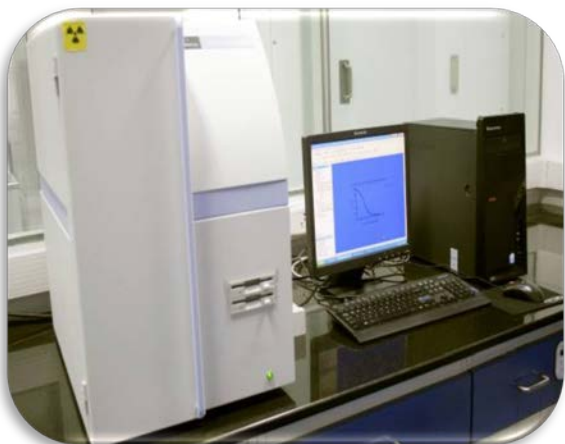
## Cell Bank:

- CHO-K1, HEK293, IMR32, Caco-2, Sf9, HepG2, U-937, and PC12.

## Recombinant stable cell line generation:

- Stable cell line are primary source for cell based assays and membrane proteins for binding assays.
- Recombinant stable cell lines were generated in CHO-K1/HEK293 parent cell line using recombinant expression construct and CRE-Luc reporter by liposomal transfection.
- Pure clones were identified by single cell per well dilution.
- Validated using reference agonist and antagonist dose response.

# Radio-ligand Binding Assays



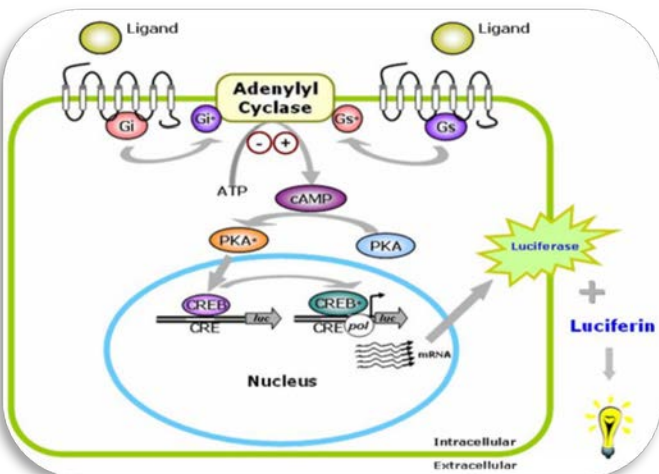
- Atomic Energy Regulatory Board of India (AERB) approved facility.
- Validated binding assays from target class GPCRs, Ion channels and Transporters
- Validated Functional uptake assays for monoamine transporters.
- Scintillation Proximity Assay (SPA) bead and filtration based platform.



# Cell Based Assays

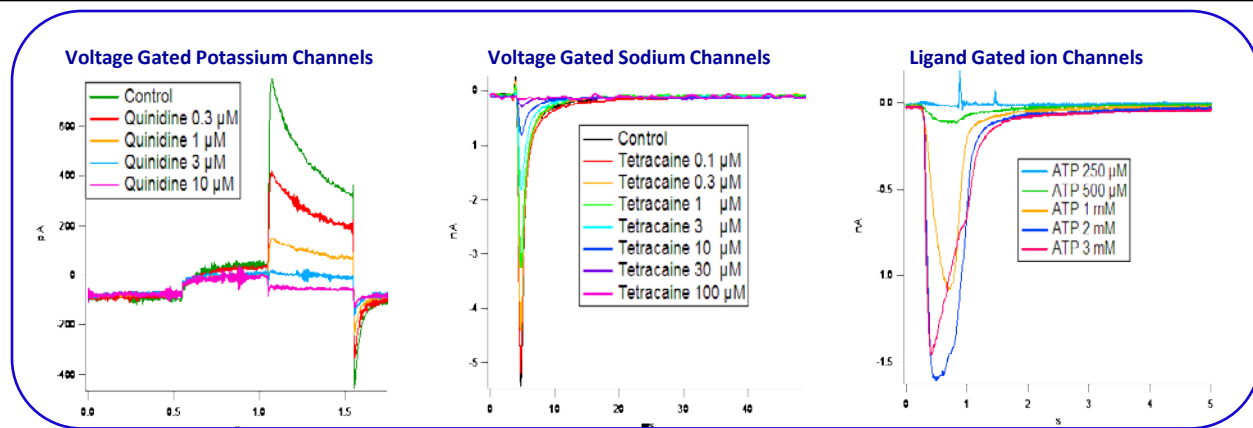


- Validated cell lines were used for screening compounds along with reference compounds in each plate.
- Luciferase reporter gene based assay was utilized to determine functionality of compounds representing various class with varying affinities.
- $EC_{50}$ ,  $IC_{50}$ , and  $K_b$  values were derived.
- Agonist, Antagonist and allosteric modulators can be identified.





# In Vitro Electrophysiology Assays



- Versatile automated Patch clamp assays for both Voltage gated and Ligand gated ion channels using Nanion Patch clamp instrument.
- Patch clamp assays for temperature sensitive receptors like TRPV1, hERG etc can be measured at ideal temperatures with inbuilt temperature control system.
- Validated assays for safety profiling and screening therapeutic targets like pain and other CNS disorders.
- Voltage gated ion channels - Potassium channels **K<sub>v</sub> 10.1(eag1)**, **K<sub>v</sub> 11.1(hERG)**; Sodium channels **Na<sub>v</sub> 1.4**, **Na<sub>v</sub> 1.5**, **Na<sub>v</sub> 1.6**, **Na<sub>v</sub> 1.7**.
- Ligand gated ion channels - Purinergic receptors **P2X7**.



# List of *In Vitro* Assays

Target	Assay Type
<b>GPCRs</b>	
Adenosine A <sub>2A</sub>	Reporter gene Driven cell based
Adrenoceptor $\alpha_{1B}$	Reporter gene Driven cell based/ Radioligand binding
Adrenoceptor $\alpha_{2C}$	Reporter gene Driven cell based
Serotonin 5-HT <sub>1A</sub>	Reporter gene Driven cell based/ Radioligand binding/ $\beta$ -arrestin
Serotonin 5-HT <sub>2A</sub>	Reporter gene Driven cell based/ Radioligand binding/ $\beta$ -arrestin
Serotonin 5-HT <sub>2C</sub>	Radioligand binding
Serotonin 5-HT <sub>4</sub>	Reporter gene Driven cell based/ Radioligand binding
Serotonin 5-HT <sub>6</sub>	Reporter gene Driven cell based
Serotonin 5-HT <sub>7</sub>	Reporter gene Driven cell based/ Radioligand binding
Dopamine D <sub>1</sub>	Reporter gene Driven cell based
Dopamine D <sub>2</sub>	Reporter gene Driven cell based/ Radioligand binding/ $\beta$ -arrestin
Dopamine D <sub>3</sub>	Radioligand binding
Dopamine D <sub>5</sub>	Reporter gene Driven cell based
Histamine H <sub>1</sub>	Reporter gene Driven cell based/ Radioligand binding



# List of *In Vitro* Assays

Target	Assay Type
<b>GPCRs</b>	
Histamine H <sub>3</sub>	Reporter gene Driven cell based
Histamine H <sub>4</sub>	Reporter gene Driven cell based/ Radioligand binding
Muscarinic M <sub>1</sub>	Reporter gene Driven cell based/ Radioligand binding/ $\beta$ -arrestin/ IP1 assay
Muscarinic M <sub>2</sub>	Reporter gene Driven cell based/ Radioligand binding
Muscarinic M <sub>3</sub>	Reporter gene Driven cell based/ Radioligand binding
Muscarinic M <sub>4</sub>	Reporter gene Driven cell based/ Radioligand binding
Muscarinic M <sub>5</sub>	Reporter gene Driven cell based/ Radioligand binding
Cannabinoid CB <sub>1</sub>	Reporter gene Driven cell based
Cannabinoid CB <sub>2</sub>	Reporter gene Driven cell based/ Radioligand binding
Prostaglandin EP2	Reporter gene Driven cell based
<b>Transporters</b>	
SERT	Radioligand binding/ Functional uptake
DOPT	Radioligand binding/ Functional uptake
NET	Radioligand binding/ Functional uptake



# List of *In Vitro* Assays

Target	Assay Type
<b>Nuclear receptors</b>	
LXR alpha	Reporter gene Driven cell based
LXR beta	Reporter gene Driven cell based
RXR alpha	Reporter gene Driven cell based
PXR	Reporter gene Driven cell based
PPAR gamma	Reporter gene Driven cell based
<b>Ion Channels</b>	
Nicotinic $\alpha 4\beta 2$	Radioligand binding
Serotonin 5-HT <sub>3</sub>	Radioligand binding
Potassium K <sub>v</sub> 10.1	Electrophysiology
Potassium K <sub>v</sub> 11.1 (hERG)	Radioligand binding/ Electrophysiology
Sodium Na <sub>v</sub> 1.4	Electrophysiology
Sodium Na <sub>v</sub> 1.5	Electrophysiology
Sodium Na <sub>v</sub> 1.6	Electrophysiology
Sodium Na <sub>v</sub> 1.7	Electrophysiology
Purinergic P2X7	Electrophysiology



- Independent Quality Assurance team
- Quality System Procedures (QSP's) for Quality System Management and Standard Operating Procedures (SOP's) for Operation, Calibration, Maintenance of Equipment's
- Document and Data Control, Conducting Internal Audits, Study Specific Audits
- Dedicated Archive facility for the retention of the records
- Facility audited and approved by many global pharmaceutical companies and majority of Indian Pharma Companies



# Contacts:

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